Week 1: 8. June 2015 – 12. June 2015

8. June 2015:

1) Amplification of *hxlA*, *hxlB* and *medh2* for TOPO cloning.
   - Pipetting scheme and PCR program according to PCR with Phusion-HF DNA Polymerase Protocol
   - Primer: hxlA_P1/P2; hxlB_P1/P2; medh2_P1/P2
   - Template: Genomic DNA from *Bacillus methanolicus* [100 ng/µl]

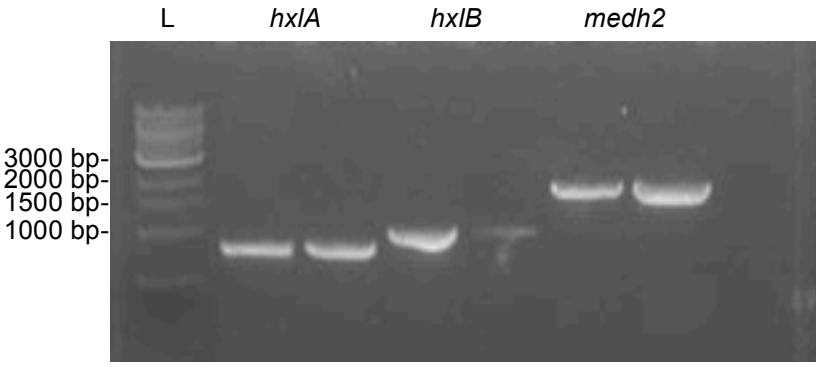

Figure 1: Amplification of *hxlA*, *hxlB* and *medh2* for TOPO cloning. 20 µl of PCR were checked on 1% (w/v) agarose gel. Expected sizes: *hxlA*-826 bp, *hxlB*-988 bp, *medh2*-1936 bp. As ladder (L) 1kB Ladder (NEB) was used.

2) Liquid culture for growth of Methylococcus capsulatus (Bath)
   - Inoculate a 30 ml culture of Methylococcus capsulatus Bath
   - Use 22.5 ml NMS media and add 7.5 Methanol as a carbon source
   - Incubate for 4 days at 37 °C

9. June 2015

1) Purification of generated *hxlA*, *hxlB* and *medh2* PCR products for TOPO cloning
   - Using Wizard® SV Gel and PCR Clean-Up System (Promega) and following the provided manual.
2) TOPO cloning of *hxlA*, *hxlB* and *medh2* into pCR4 Vector
   - Using the Zero Blunt Topo cloning Kit (Invitrogen)
   - See TOPO cloning protocol for pipetting scheme
3) Transformation of *E. coli* TOP10 cells with TOPO cloning reactions
   - Add 2 µl of TOPO reaction to chemically competent *E. coli* TOP10 cells.
   - Following the protocol for transformation of chemical competent *E. coli* cells
   - Plate on LB+Amp [100 µg/ml] and incubate overnight at 37 °C

10. June 2015

- Transformation was successful and colonies were grown on the plate.

1) Colony-PCR to screen for clones containing the TOPO constructs
   - Constructs: pCR4+*hxlA*, pCR4+*hxlB*, pCR4+*medh2*
   - Pipetting scheme and PCR program according to PCR with Taq-DNA Polymerase Protocol
   - Primer: hxlA_P1/P2, hxlB_P1/P2, medh2_P1/P2
   - Check 6 Clones per construct
   - Positive Control: add 1 µl of PCR product of *hxlA*, *hxlB* and *medh2*
   - Negative Control: add 1 µl MilliQ Water

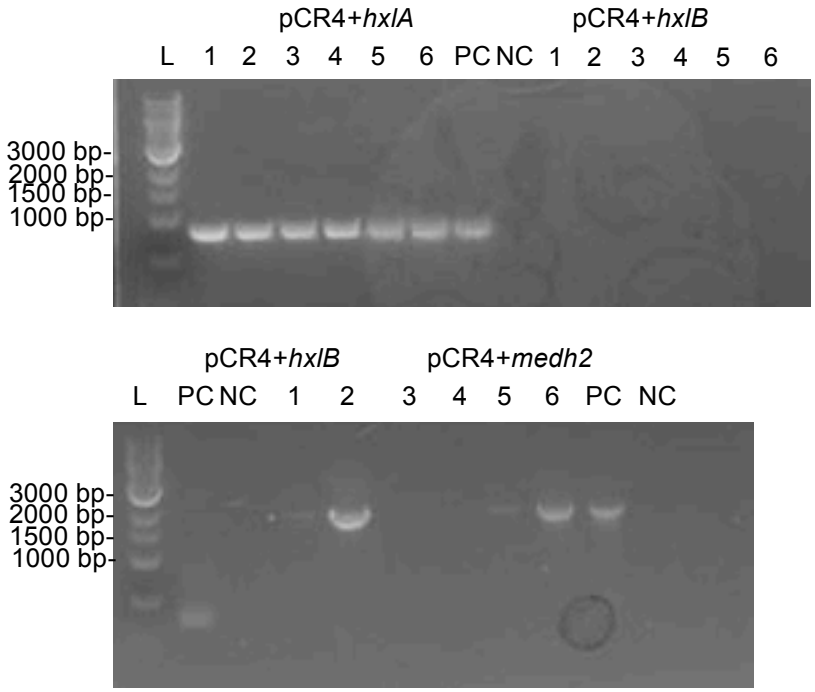

Figure 2: Colony-PCR to identify clones containing TOPO constructs. Numbers 1-6 determine the checked clone. As positive control (PC) 1 µl PCR fragment was added. As negative control (NC) 1 µl MilliQ Water was added. 10 µl of PCR were analzyed on 1 % (w/v) agarose gel. Expected sizes: *hxlA*-826 bp, *hxlB*- 988 bp, *medh2*-1936 bp. As ladder (L) 1 kB Ladder was used.

2) Inoculate liquid culture for plasmid isolation of pCR4+*hxlA*, pCR4+*hxlB* and pCR4+*medh2*
   - Inoculate clone 1 for plasmid isolation of pCR4+hxlA and clone 2 for plasmid isolation of pCR4+medh2 with 5 ml LB+Amp [100 µg/ml] and incuabte at 37 °C overnight shaking at 220 rpm.

- Although the Colony-PCR did not show positive pCR4+*hxlB* clones, we inoculated clone 3 with 5 ml LB+Amp [100 µg/ml], because also our positive control did not work, so we assumed a problem with the PCR reaction.

11. June 2015

1) Plasmid Isolation of pCR4+*hxlA*, pCR4+*hxlB* and pCR4+*medh2*
   - Use the QIAprep Spin Miniprep Kit (Qiagen) and follow the provided manual.
2) Amplification of *mmoC* and *mmoG* for TOPO cloning
   - Pipetting scheme and PCR program according to PCR with Phusion-HF DNA Polymerase Protocol
   - Primer: mmoC_P1/P2, mmoG_P1/P2
   - Template: 1 µl of *Methylococcus capsulatus* liquid culture

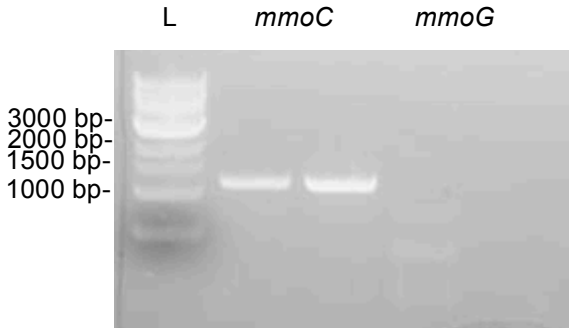

Figure 3: Amplification of *mmoC* and *mmoG* for TOPO cloning. 20 µl of PCR were checked on 1% (w/v) agarose gel. Expected sizes: *mmoC*-1142 bp and *mmoG*-2249 bp. As ladder (L) 1kB Ladder (NEB) was used.

12. June 2015

- Plasmids from iGEM2014 Team Braunschweig arrived. Each Plasmid consists of one gene encoding for the sMMO subunit (*mmoXYZBCD*) in the pSC1B3 vector backbone.

Sequencing Reactions

- Send Plasmids pCR4+*hxlA*, pCR4+ *hxlB* and pCR4+*medh2* for sequencing.
  - Correct sequence of all cloned genes confirmed.
- Send Plasmids pSC1B3+ sMMO subunit gene for sequencing.
  - pSC1B3+*mmoY* contains a point mutation changing the codon GGT into GGC. Both codons encode the aminoacid Glycin.
  - Correct sequence of all other sMMO subunits confirmed.